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Impregnating and Coating with Endrin to Protect Douglas-fir Seed from Rodents

Use Pesticides Safely
FOLLOW THE LABEL

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INTRODUCTION

Reforestation with coniferous seed is often prevented by various biota which destroy disseminated seed before germination. Although the relative importance of destructive agents varies by area, seed species, and year, seed-eating rodents are generally held responsible for the greatest seed losses in the Pacific Northwest. Various measures have been used to reduce rodent impacts and enhance reproduction from sown seed. At present, the most common protective treatment of seed is with endrin.[1/]

The U.S. Bureau of Sport Fisheries and Wildlife recommends endrin as a seed coating at 0.5 percent, with an adhesive to hold it to the seedcoat and a coloring material for identification. Although early field tests showed that adequate stocking with Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) was possible when endrin-coated seed was used (Dimock 1957, Hooven 1957, Dick et al. 1958),[2/] failures have occurred in many operational seedings. Many forest managers, therefore, have recently questioned the effectiveness of the recommended 0.5-percent treatment and have often employed much higher concentrations of endrin.

Endrin was introduced in 1956 as an interim seed protectant until a better compound became available. Since that time, emphasis has been on a search for new biologically active chemicals to replace endrin. Unfortunately, this effort has not been successful. Today, endrin remains the only chemical recommended for protecting seed from rodents; yet, only empirical guidelines for its use are available. Since the midfifties, endrin has been used in varying concentrations, with different coating methods, and in combinations with other chemical ingredients. Despite such trials, no substantial improvements in the basic treatment have either been verified or become generally accepted. Further, there is still no agreement on the effectiveness of endrin in protecting Douglas-fir seed or its effect on seed viability.

In the experiments reported here, therefore, we have attempted to evaluate endrin critically, using four seed lots, two methods of germination, and two bioassay techniques. The chemical was applied to seed by a conventional coating method at two levels, using the most common adhesive and coloring material, and by impregnation. We treated small and large batches of seed, simulated seeding by helicopter, and determined endrin content of seed from the different treatments.

[1/] 1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4, 5, 8-endo-endo-dimethanonaphthalene.

[2/] Names and dates in parentheses refer to Literature Cited, p. 17.

EXPERIMENTAL

Seed.-- Four lots of western Washington Douglas-fir seed were obtained from the Washington State Department of Natural Resources and used for testing. Available information for each lot is shown in table 1.

Table 1.--*Seed data as determined by the Washington State Department of Natural Resources just prior to storage*

Seed lot number	Year collected	Elevation	Purity	Moisture content	Number of seeds per pound
		- - Feet - -	*- - - Percent - - -*		
1	1964	1,100-1,500	97.5	5.6	44,039
2	1964	1,200-1,700	96.0	8.4	44,470
3	1965	1,200-1,600	95.3	7.5	40,504
4	1966	2,000	97.5	8.0	40,500

We used lots 1-3 in experiment 1, lot 1 in experiments 2-5, and lot 4 in experiment 6. All seeds were kept in moisture-proof containers at about -4° C. until needed.

Test animal.-- Deer mice *(Peromyscus maniculatus)*, live-trapped in a deforested site adjacent to standing second-growth Douglas-fir timber about 1 mile southeast of Tumwater, Wash., were used in all bioassay tests. All mice were trapped from October through April when Douglas-fir seed is usually available. The animals were brought to the laboratory, placed in individual cages, and supplied with water and commercial pelleted ration until tests were conducted.

Seed treatments.-- Seeds were treated as follows:

1. *Control:* Seed was not chemically treated.
2. *Endrin-coated:* Endrin 50-WP (50-percent wettable powder) from Stauffer Chemical Company[3/] was used. Seed was wet

[3/] Mention of chemical companies and their products does not represent endorsement by the Forest Service or by the Department of Agriculture.

with a slurry of endrin in Dow Latex 512-R adhesive, covered with aluminum powder, spread in a thin layer, and allowed to dry overnight under a fume hood. The two coating treatments used in the tests were:

a. *0.5-percent endrin* (0.5 EC): Endrin was added at the 0.5-percent level as recommended by the U.S. Bureau of Sport Fisheries and Wildlife.

b. *1.0-percent endrin* (1.0 EC): Endrin concentration was double the recommended rate. Seed treated at this higher concentration is now used by many foresters in operational direct-seedings.

Endrin-coated seed was prepared in 1/2-pound batches in glass jars, except in experiment 6 where 10 pounds of seed were coated in a cement mixer in a typical commercial manner.

3. *Endrin-impregnated:* Seed was dried in a forced-air oven at 40° C. for 24 to 48 hours to reduce moisture content to about 6 percent prior to impregnation. Preliminary experiments showed that this pretreatment does not affect viability of Douglas-fir. Also, as with seed treated with other chemicals (Jones and Havel 1968), our unpublished data showed that a low moisture content was necessary to minimize injury to seed during impregnation.

Effects of several endrin solvents on viability of Douglas-fir seed were studied in preliminary experiments. For the impregnation studies reported here, we selected the two solvents which appeared most promising.

Solutions containing 2 percent endrin were prepared by dissolving appropriate amounts of Endrin 50-WP in each of the two selected solvents and filtering out the inactive, insoluble ingredients of the wettable powder. Dried seeds were then soaked in the endrin solutions for predetermined time periods and were finally dried in a forced-air oven at 40° C. for 24 hours to remove the solvent from the seed without affecting the endrin.

The two endrin-impregnation treatments used in the tests were:

a. *Endrin-dichloroethane* (EDI): Endrin was dissolved in 1,2-dichloroethane (D).

b. *Endrin-trichloroethylene* (ETI): Endrin was dissolved in trichloroethylene (T).

In experiment 6, 10 pounds of seed were treated in a special impregnation container designed to handle up to 14 pounds of seed. In all other experiments where impregnated seed was used, the needed small amounts of seed were treated by enclosing seeds in cheesecloth and dipping them in solutions contained in beakers.

4. *Solvent-impregnated:* To separate effects of endrin from those of the solvent, dried seed was soaked in each of the two selected solvents in the same manner and for the same periods of time as were the small batches of endrin-impregnated seed. This seed was also dried at 40° C. for 24 hours after treatment. The solvent-impregnated treatments were:

   a. *Dichloroethane* (DI): Seed was soaked in the (D) solvent.

   b. *Trichloroethylene* (TI): Seed was soaked in the (T) solvent.

Additional information about the treatments is given with each experiment as required.

Germination.--Four 100-seed replicates were germinated on perlite at 24° ± 1° C. after stratification for 21 days at 3° to 5° C., as prescribed in the standard test (Association of Official Seed Analysts 1965). Germinants were counted at weekly intervals for 4 weeks.

Production of seedlings and seedling growth were also studied concurrently with the perlite germination tests. Two additional 100-seed replicates of stratified seed were planted about 1/8-inch deep in 4- by 4-inch pots containing equal amounts of soil, vermiculite, and peat moss. For germination and initial growth, pots were placed in a plant growth chamber and watered daily. The chamber was set for 27° C. and 800 foot-candles (ft.-c.) of light for 20 hours per day and 21° C. for 4 hours of darkness; relative humidity was about 70 percent. At the end of 28 days, the pots were transferred to another chamber and watered every 2 days. Temperatures were 27° C. during the day and 17° C. at night. Other growth conditions were 60- to 80-percent relative humidity and 1,200 ft.-c. of fluorescent-incandescent light on a 14-hour photoperiod. Seedlings were harvested and counted 50 days after planting. Roots were washed, severed at the root collar, and discarded after examination. The tops of 10 seedlings, selected at random from each pot, were cut into small segments and dried to constant weight in a forced-air oven at 65° C.

Bioassay.-- Bioassays were conducted whenever the number of mice caught was sufficient for a minimum of one test. Tests, therefore, were conducted at different times, but each test was run with freshly caught mice. One day prior to each test, mice, in individual cages supplied with pelleted food and water, were offered a number of Douglas-fir seed equal to that subsequently used in the test; animals not consuming at least 90 percent of the untreated seeds were rejected. Treatments were assigned at random to remaining mice. Water and food were available *ad libitum*.

Number of mice per treatment, number of seed offered each test day, and duration of tests varied in the four experiments involving mice. Thus, we used 12 mice and 80 seeds per day per mouse for 2 consecutive days in experiment 2 and the same conditions with washed seed in experiment 4; a 10-animal, 40-shelled seeds, 2-day test in experiment 4; and a 10-animal, 10-seed, 5-day test in experiments 5 and 6. Seed consumption was recorded daily, and the numbers of dead mice were noted each day during the test and for 2 days following.

Endrin content.-- Endrin was extracted from seed by a double extraction technique to ensure maximum recoveries. Duplicate samples of 200 seeds each were pulverized in a Virtis homogenizer with 50-ml. portions of Skellysolve B:isopropyl alcohol (4:1 by volume). Extracts were separated from the slurry by filtration, and residual seed material was reextracted with Skellysolve B for 20 hours in Soxhlet apparatus. Extracts were combined and evaporated to dryness in a rotary vacuum evaporator. Extracted fats were saponified by adding 25-ml. portions of 95-percent ethyl alcohol:50-percent potassium hydroxide (4:1 by volume) to the residues and refluxing for 2 hours on a hot plate. The saponified solutions were cooled, diluted with equal volumes of water, and extracted with four 20-ml. portions of Skellysolve B. Skellysolve B extracts containing the endrin were combined and washed with water until neutral to phenolphthalein. Extracts were then dried over anhydrous sodium sulfate, made up to volume with hexane, and analyzed for endrin.

Amounts of endrin in the extracts were determined by gas chromatography using an Aerograph Model 1840-1 instrument equipped with an electron capture detector and a 5-foot by 1/8-inch I. D. glass column packed with 6-percent SE-30 on 80- to 100-mesh Varaport-30. Injector, column, and detector temperatures were 200°, 190°, and 200° C., respectively. Nitrogen was used as carrier gas at a flow rate of 40 ml. per minute. Endrin was quantified by peak height, and data were appropriately corrected according to recovery analyses of known quantities of endrin added to blank seed samples.

Experiment 1

Using three seed lots, we studied effects of two endrin coatings, two impregnations with endrin solvents, and two endrin impregnations on seed germination and ability to produce normal seedlings. With the solvent- and endrin-impregnations, we also determined effects of five different soaking times. Because of limited germination facilities, we were unable to evaluate all treatments simultaneously and the experiment, therefore, consisted of five separate tests. Each test included a control, the two endrin coating treatments, and one soaking time period for each of the four solvent- and endrin-impregnation treatments. Soaking periods were 1/2, 1, 1-1/2, 2, and 2-1/2 hours in tests 1, 2, 3, 4, and 5, respectively. In addition, coated and impregnated seeds were freshly prepared for each test, and control seed was always drawn from cold storage immediately before testing.

Results.-- Germination of untreated seed was almost always better on perlite than in soil (table 2). In both media, seed performance differed among lots, with germination highest in lot 1, intermediate in lot 2, and lowest in lot 3. Also, average dry weights of individual seedling tops from untreated seed varied slightly by lot, but trends were not consistent between tests.

Gross comparisons of average dry weights of seedling tops and appearance of the roots indicate that seedling growth was not affected by treatment. Germination data, however, show that treatment affected the seed. Effects varied among treatments and, in most, germination percents in soil were

Table 2.-- *Germination of treated and untreated Douglas-fir seed on perlite and in soil, and ovendry weight of seedling tops produced in soil*

Seed treatment[1]	Seed lot 1			Seed treatment[1]	Seed lot 2			Seed treatment[1]	Seed lot 3		
	Perlite	Soil	Dry weight[2]		Perlite	Soil	Dry weight[2]		Perlite	Soil	Dry weight[2]
	Percent[3]		*mg.*		*Percent*[3]		*mg.*		*Percent*[3]		*mg.*
					TEST 1						
Control	90	81	31	Control	86	78	26	Control	78	69	25
DI	86	80	30	EDI	84	78	27	EDI	75	78	31
EDI	86	84	30	DI	82	82	29	1.0 EC	74	64	27
0.5 EC	82	80	30	TI	81	70	28	0.5 EC	74	70	33
1.0 EC	82	82	26	ETI	80	72	24	DI	73	74	32
TI	80	84	32	1.0 EC	79	80	33	TI	72	70	25
ETI	78	76	31	0.5 EC	74	79	31	ETI	68	65	32
					TEST 2						
Control	88	79	29	Control	83	78	24	0.5 EC	82	74	26
DI	88	88	25	DI	81	86	28	Control	78	76	22
0.5 EC	86	83	32	EDI	80	78	34	DI	74	76	30
EDI	82	84	29	1.0 EC	79	82	28	EDI	72	74	33
TI	82	84	26	TI	78	80	30	TI	72	70	33
1.0 EC	81	85	25	0.5 EC	77	79	27	ETI	65	74	26
ETI	76	84	30	ETI	75	76	25	1.0 EC	65	76	33
					TEST 3						
Control	90	85	32	Control	88	87	33	0.5 EC	80	78	29
0.5 EC	88	72	31	0.5 EC	84	80	28	Control	80	76	29
DI	86	89	33	1.0 EC	83	76	26	1.0 EC	79	75	28
1.0 EC	82	81	29	DI	82	87	31	DI	74	84	33
EDI	81	90	32	EDI	80	84	33	EDI	72	85	27
TI	72	76	30	TI	70	76	35	TI	62	66	33
ETI	70	75	33	ETI	66	72	31	ETI	58	66	31
					TEST 4						
Control	89	83	27	Control	83	85	27	Control	81	77	26
1.0 EC	88	85	29	0.5 EC	82	86	25	DI	79	84	23
0.5 EC	85	82	28	EDI	80	84	24	0.5 EC	78	78	26
DI	84	87	24	DI	80	82	29	1.0 EC	75	74	29
EDI	83	92	24	1.0 EC	78	84	30	EDI	75	77	26
TI	60	76	24	ETI	61	78	27	ETI	57	64	29
ETI	58	78	31	TI	54	72	23	TI	53	64	28
					TEST 5						
0.5 EC	89	83	25	0.5 EC	84	74	27	Control	80	76	25
Control	88	86	22	Control	82	82	20	1.0 EC	80	79	27
DI	83	88	26	1.0 EC	82	81	24	0.5 EC	78	75	27
EDI	82	84	25	DI	81	90	21	DI	73	77	22
1.0 EC	82	82	25	EDI	80	82	27	EDI	70	80	23
TI	54	74	24	TI	51	67	22	TI	40	67	26
ETI	52	74	22	ETI	44	71	24	ETI	38	66	26

[1] Control = untreated, 0.5 EC = endrin-coated at 0.5 percent, 1.0 EC = endrin-coated at 1.0 percent, DI = dichloroethane-impregnated, TI = trichloroethylene-impregnated, EDI = endrin-dichloroethane-impregnated, and ETI = endrin-trichloroethylene-impregnated.

For solvent- and endrin-solvent-impregnation treatments, seed was soaked for ½, 1, 1½, 2, and 2½ hours in tests 1, 2, 3, 4, and 5, respectively. Impregnated and coated seeds were freshly prepared for each test.

[2] Ovendry weights of tops per seedling are averages of 20 seedlings, 10 tops from each of two pots.

[3] Germination percents are averages of four and two replications each for perlite and soil tests, respectively. Within each test, means in the perlite column enclosed by the same line(s) do not differ significantly at the 5-percent level, using Tukey's test (Snedecor 1961).

higher than on perlite. This trend is opposite that of untreated seed but agrees with performance of many seed lots recently reported by Stein (1967).

Endrin coatings significantly reduced germination on perlite in two comparisons. Also, two germinations in soil from coated seed were less than the controls by more than 10 percent.

Performance of EDI seed was similar to that of coated seed on perlite and in soil and compared well with germination of the controls in soil. However, EDI seed germination on perlite was significantly lower than that of DI and control seed in about 20 percent of the comparisons. Also, its germination in soil in two comparisons was 8 percent lower than that of the DI seed.

Performance of ETI seed was not as good as that of the EDI seed especially when seed was soaked for more than 1 hour. Its germination on perlite was significantly lower than that of TI seed in about 20 percent of the comparisons and was mostly inferior to that of seed from the other treatments. With few exceptions, germination of ETI seed in soil was higher than on perlite. Thus, performance of this seed in soil was comparable with that of TI seed and was only somewhat lower than that of seed from the other treatments in most comparisons.

Experiment 2

Using seed from lot 1, this bioassay was conducted to compare effects of two coating and four impregnation treatments on seed consumption by deer mice. The coating treatments, 0.5 EC and 1.0 EC, are typical of those currently employed in the Pacific Northwest. The impregnation treatments involve use of two endrin solvents and soaking times of 1 hour (EDIa and ETIa) and 2-1/2 hours (EDIb and ETIb), as these appeared to provide measurable treatment effect.

Seed was bioassayed in a 12-animal, 80-seeds per animal per day, 2-day test. The 80-seed offering was selected after preliminary feeding trials showed that virtually all mice would readily eat 80 untreated seeds per day and many would eat 80 or more treated seeds of at least one of the treatments.

Results.-- All treatments reduced feeding (table 3). Although significantly better than the control, the 0.5 EC was only about half as effective as any of the other treatments. Increasing the concentration from 0.5 to 1.0 EC greatly improved effectiveness of the treatment.

The tests revealed no differences in feeding by mice on impregnated seeds which were all as effective as the 1.0 EC. Endrin impregnation with either the D or T solvent gave similar reductions in feeding, but individual consumption was somewhat more variable among mice fed the ETI treatments. Increasing the soaking time from 1 to 2-1/2 hours gave no apparent benefit.

Mortality resulted from all endrin treatments, and highest mortality occurred with impregnation and 1.0 EC treatments. Again, doubling the concentration from 0.5 to 1.0 EC nearly doubled mortality. Mortality was similar within the 1-hour impregnation treatments but was somewhat less when the soaking time was increased to 2-1/2 hours.

Table 3.--*Consumption of endrin-treated Douglas-fir seed by deer mice and resulting animal mortality*

Treatment[1/]	Seed consumption[2/]		Animal mortality
	- Mean number -	*- - - Percent - - - -*	
Control	160a	100	0
0.5 EC	96b	60	42
ETIa	48c	30	92
1.0 EC	48c	30	92
ETIb	45c	28	75
EDIb	41c	26	67
EDIa	40c	25	92

[1/] Control = untreated, 0.5 EC = endrin-coated at 0.5 percent, 1.0 EC = endrin-coated at 1.0 percent, EDIa = endrin-dichloroethane-impregnated for 1 hour, EDIb = endrin-dichloroethane-impregnated for 2½ hours, ETIa = endrin-trichloroethylene-impregnated for 1 hour, ETIb = endrin-trichloroethylene-impregnated for 2½ hours.

[2/] 12-animal, 80-seeds per animal per day, 2-day test.

[3/] Means followed by the same letter do not differ significantly at the 5-percent level, using Tukey's test (Snedecor 1961).

Experiment 3

The biological activity of endrin-treated seed against mice depends on its endrin content and the distribution of the chemical on and in the seedcoat and within the seed. In this experiment, therefore, we determined endrin content of: (1) seed coated with endrin at the recommended 0.5-percent level, (2) 1.0-percent endrin-coated seed similar to that used by many foresters, and (3) endrin-impregnated seed, EDIa, which showed satisfactory germination in experiment 1 and biological activity equal to that of the 1.0 EC seed in experiment 2. Further, we washed EDIa seed repeatedly with water to remove surface endrin, shelled the washed seed (EDIa-w), and determined the endrin content of resulting seedcoats and "endosperms."[4/] Seed from lot 1 was used throughout the experiment.

Results.--Because endrin losses are inherent in the seed-coating method, 0.5 EC and 1.0 EC seed contained only 62 and 83 percent, respectively,

[4/] "Endosperm" denotes all material inside the seedcoat.

Table 4.--*Endrin content of coated and impregnated Douglas-fir seed*

Treatment[1/]	Seed part analyzed	Endrin content	
		µg.[2/]	*Percent*
0.5 EC	Whole seed	32.0	0.31
1.0 EC	Whole seed	85.5	.83
EDIa	Whole seed	40.0	.39
EDIa-w	Seedcoat	22.0	--
EDIa-w	"Endosperm"	4.1	--

[1/] 0.5 EC = endrin-coated at 0.5 percent, 1.0 EC = endrin-coated at 1.0 percent, EDIa = endrin-dichloroethane-impregnated for 1 hour, EDIa-w = same as EDIa but treated seed was washed 10 times with water.

[2/] Determined by gas chromatography; each value is the average of two determinations.

of the actual amounts of endrin applied to them. Endrin content of whole EDIa seed was less than half that in the 1.0 EC seed, 25 percent more than in seed from the 0.5 EC treatment, and 22 percent less than the recommended 0.5-percent level (table 4). Unlike coated seed where endrin was limited to seed surfaces, impregnated seed contained endrin on the surface, in the seedcoat, and in the "endosperm." Also, total endrin content of EDIa seed after washing indicates that most of the endrin was closely tied to the impregnated seed.

Experiment 4

After being sown in the field, endrin-impregnated seed would likely lose some endrin by weathering. To evaluate the effect of such losses, we bioassayed washed endrin-impregnated seed (EDIa-w) similar to that used in experiment 3. We also shelled washed seed and bioassayed the "endosperms" to determine the protective value of endrin inside the seed. We used 10-animal, 2-day tests with 80 washed seeds and 40 "endosperms" per animal per day. Fewer "endosperms" were offered because of the difficulty in removing seedcoats to obtain the test material.

Results.--Consumption of EDIa-w seed and "endosperms" was considerably less than untreated seed (table 5). Percents of washed seed eaten were similar to those of the 1.0 EC and all the endrin-impregnated seed consumed in experiment 2. On the other hand, percent of "endosperms" eaten was higher, but the actual number taken was nearly the same as for the 1.0 EC and impregnated seed in experiment 2.

Table 5.--*Consumption of whole seed and "endosperm" of washed endrin-impregnated seed by deer mice and resulting animal mortality*

Treatment[1/]	Seed part bioassayed	Seed consumption[2/]		Animal mortality
		Mean number	- - - *Percent* - - - -	
Control	Whole seed	160± 1	100	0
EDIa-w	Whole seed	43±11	27	80
EDIa-w	"Endosperm"	39± 2	49	60

[1/] Control = untreated, EDIa-w = endrin-dichloroethane-impregnated seed (1 hour) washed 10 times with water to remove surface endrin.

[2/] 10-animal, 80-seeds per animal per day, 2-day test for whole seed and 10-animal, 40-"endosperms" per animal per day, 2-day test for "endosperm." Mean numbers are followed by confidence intervals (P = 0.05).

Consumption of both washed seed and "endosperms" resulted in death of sizable proportions of test animals. This mortality and the reduced seed consumption indicate that impregnated seed would probably retain its effectiveness in the field despite weathering.

Experiment 5

The previous experiments showed that among the endrin-impregnations tested, the EDIa treatment was most promising. To substantiate earlier results and to investigate the main treatments further, we bioassayed EDIa seed again together with seed from the two coating treatments in the fall of 1968. Lot 1 seed was used as in earlier tests, but a different testing procedure was followed. With the 80-seed test used in experiment 2, most of the treated seed consumed was taken the first day of the 2-day test period presumably because the animals had ample opportunity to rapidly ingest greater numbers of seed than needed for aversion or mortality. In this experiment, therefore, we used a 10-animal, 10-seeds per animal per day, 5-day test in order to measure treatment effects over a longer period of time.

Results.--As in experiment 2, consumption of 1.0 EC and EDIa seed was markedly lower than that of untreated seed (table 6). However, unlike the earlier test (table 3), the 0.5 EC treatment did not reduce feeding significantly. In addition, percents of seed consumption with all treatments were

Table 6.--*Consumption of endrin-treated Douglas-fir seed by deer mice and resulting animal mortality*

Treatment[1/]	Seed consumption[2/]		Animal mortality
	Mean number[3/]	- - - - *Percent* - - - - -	
Control	50a	100	0
0.5 EC	44a	88	30
EDIa	23b	46	80
1.0 EC	20b	40	50

[1/] Control = untreated, 0.5 EC = endrin-coated at 0.5 percent, 1.0 EC = endrin-coated at 1.0 percent, EDIa = endrin-dichloroethane-impregnated for 1 hour.

[2/] 10-animal, 10-seeds per animal per day, 5-day test.

[3/] Means followed by the same letter do not differ significantly at the 5-percent level, using Tukey's test (Snedecor 1961).

higher than before, and with most treatments some seed was taken on each day of the 5-day test (fig. 1).

Mortality of test animals resulted from all endrin treatments as in experiment 2. Mortality percents, however, were lower than before, particularly with the coating treatments.

Experiment 6

Operational seedings with endrin-treated seed require treatment of 10- to 25-pound batches of seed, which are then sown by helicopter. Endrin content and biological activity of the seed immediately after treatment may differ from seed treated in small batches as in the previous experiments. Also, the same parameters may be reduced as treated seed passes through the helicopter's seed dissemination equipment. To test these possibilities, we divided 20 pounds of seed from lot 4 into two equal parts. Ten pounds were coated with endrin at the 1.0-percent level in a cement mixer, and the remainder was impregnated by the EDIa treatment using the apparatus shown in figure 2. One-pound samples of each treatment were run through the seeding equipment of a grounded helicopter; equipment was set at approximately the same speed usually used in actual seedings in the field. Subsamples of coated and impregnated seed, before and after the helicopter treatment, were then analyzed for endrin by gas chromatography and fed to deer mice in a 10-animal, 10-seeds per animal per day, 5-day test.

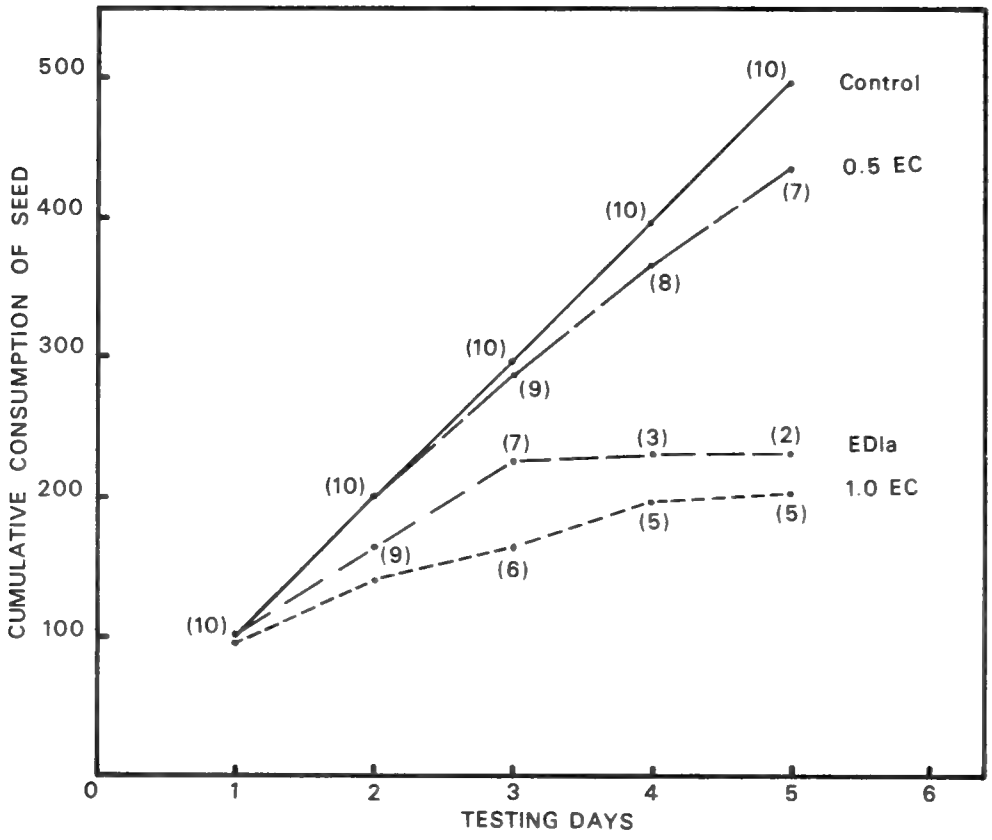

Figure 1.--Daily consumption of control and endrin-treated Douglas-fir seed by deer mice. (Numbers of surviving mice indicated in parentheses. Control = untreated, 0.5 EC = endrin-coated at 0.5 percent, 1.0 EC = endrin-coated at 1.0 percent, EDIa=endrin-dichloroethane-impregnated for 1 hour).

Results.-- Endrin contents of the 1.0 EC and EDIa seed (table 7) were similar to those obtained with seed treated in small batches in experiment 3 (table 4), although the endrin content of individual seed averaged slightly more than before probably due to differences in number of seed per pound between the lots used in the two experiments (table 1). Consumption by mice of coated and impregnated seed was, respectively, equal to and somewhat less than with the same two treatments in experiment 5 (table 6), and mortality of test animals in each treatment in the two experiments varied only slightly. Also, as in experiment 5, there was no significant difference in seed consumption between treatments, and mortality resulting from impregnated seed was higher than from the coating treatment.

Endrin content of coated and impregnated seed decreased slightly after helicopter dissemination, but the decrease was less for impregnated seed. Similarly, consumption of disseminated seed from each of the two treatments did not change significantly. However, mortality was somewhat higher among mice fed the coated seed and lower among those fed the impregnated seed after helicopter dispersal.

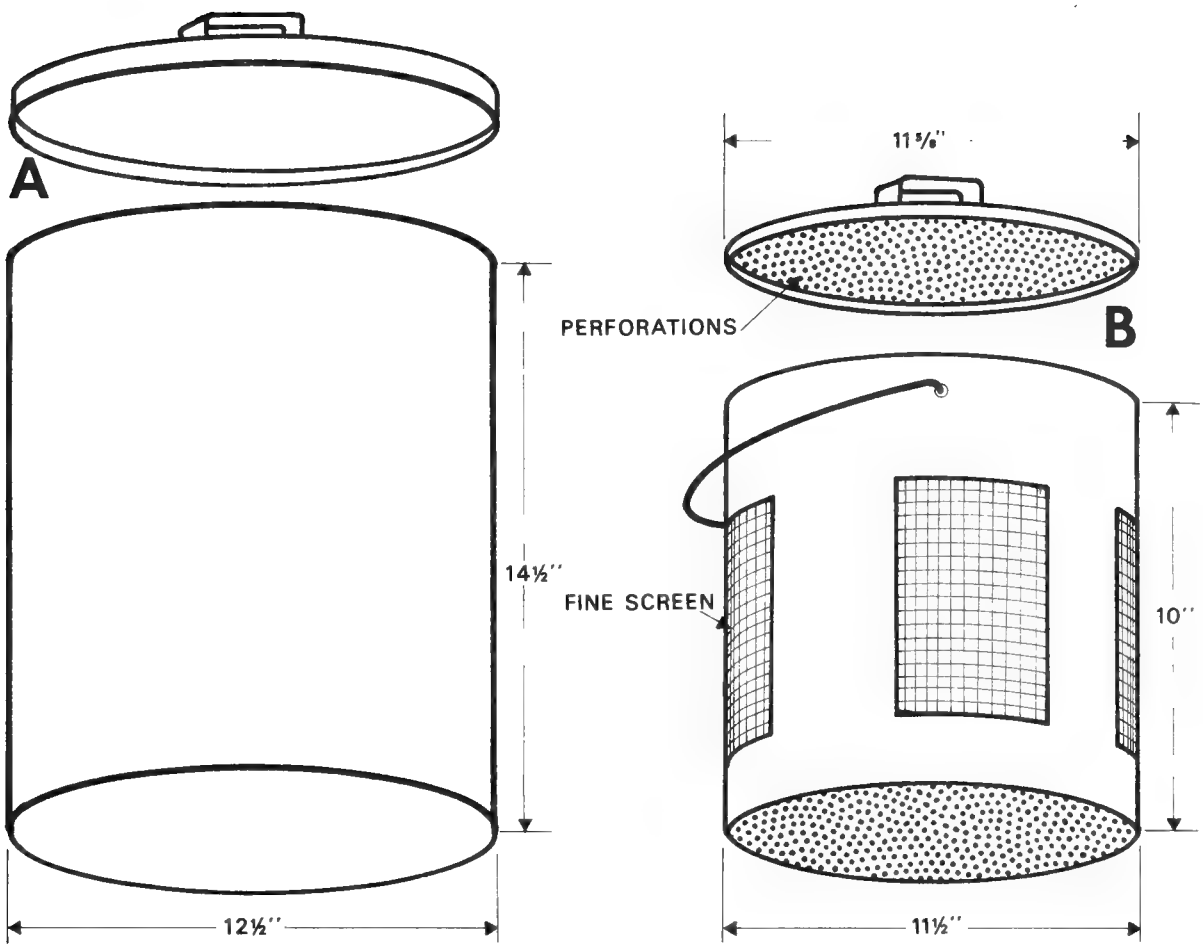

Figure 2.--Schematic diagram of apparatus used to impregnate seed in experiment 6. The apparatus, made of galvanized steel, consists of an outside container (A) to retain the endrin solution and an insert (B) to hold the seed.

Table 7.--*Endrin content and consumption by deer mice of coated and impregnated Douglas-fir seed and resulting animal mortality before and after dispersal by helicopter*

Treatment[1/]	Endrin content				Seed consumption[2/]				Animal mortality	
	Before	After	Before	After	Before		After		Before	After
	μg. per seed[3/]		*Percent*		*Mean number*	*Percent*	*Mean number*	*Percent*	*Percent*	
1.0 EC	93.5	82.5	0.83	0.73	20a	40	19a	38	40	60
EDIa	51.0	47.0	.45	.42	15a	30	17a	34	90	70

[1/] 1.0 EC = endrin-coated at 1.0 percent in a commercial cement mixer; EDIa = endrin-dichloroethane-impregnated for 1 hour using apparatus shown in figure 2.

[2/] 10-animal, 10-seeds per animal per day, 5-day test. Means followed by the same letter do not differ significantly at the 5-percent level, using Tukey's test (Snedecor 1961).

[3/] Determined by gas chromatography; each value is the average of two determinations.

DISCUSSION

With few exceptions, endrin coating at both the 0.5- and 1.0-percent level produced satisfactory germinants on perlite and seedlings in soil. Several laboratory investigations showed germination inhibitions up to 20 percent after endrin treatment (Dimock 1957, Dick et al. 1958, Edgren 1968). However, we believe that slight inhibitions are unimportant in view of the inherent inaccuracy of all laboratory results when extrapolated to the field and because of the anticipated seed protection by the treatment. Likewise, we do not believe that the high inhibitions reported were due to endrin. Rather, we suggest that the inhibitions were caused by other factors, such as method of treatment, delay in testing germination after treatment without adequate drying of the seed, or chemical ingredient other than those used in our experiments.

Germination and seedling production from seed impregnated with endrin depended on the solvent used. Performance of seed treated with the (D) solvent alone or in combination with endrin was satisfactory and considerably better than that of the TI and ETI seed. It is evident, therefore, that 1,2-dichloroethane (Ethylene dichloride) is the preferred solvent for impregnating Douglas-fir seed with endrin and possibly with other chemical protectants. The solvent may also be useful in impregnating other conifer seed since our unpublished data[5/] indicate that seed of ponderosa pine *(Pinus ponderosa* Laws.) and longleaf pine *(Pinus palustris* Mill.) are not adversely affected by the treatment. Additional advantages of the solvent are: commercial availability at reasonable cost, relative nontoxicity to mammals, low flammability, and nonpersistency since it is easily evaporated at moderately low temperatures.

Endrin content of coated seed varied among the treatments. When seed was treated in small batches, the 0.5- and 1.0-percent coated seed contained 0.31 and 0.83 percent endrin, respectively. Similarly, seed from the 10 pounds treated at the 1.0-percent level contained 0.83 percent endrin. Clearly, the coating treatments did not allow application of all endrin added to the seed. This is probably true with all coating treatments. The endrin content of seed treated with the same amount of the chemical will always vary depending mainly on kind and level of inactive ingredients added, equipment used, and accuracy in measuring the endrin and treating the seed.

Endrin content of 1.0 EC seed decreased slightly by helicopter dissemination, but seed consumption by mice did not change significantly. Whether similar results would be obtained with other commercially coated seed and other helicopters with different disseminators is not known.

The EDIa seed prepared in experiments 3 and 6 contained 0.39 and 0.45 percent endrin, respectively. The average endrin content of this impregnated seed, therefore, was somewhat lower than the recommended 0.5-percent level and approximately half that found on the 1.0-percent coated seed. The

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5/ Data on file at the Forestry Sciences Laboratory, Olympia, Wash.

chemical was distributed on and in the seedcoat and in the "endosperm." Highest endrin concentrations were in seedcoats, indicating a tendency for accumulation of the chemical in this tissue. Although amounts of endrin in "endosperms" were very small, the mere presence of the chemical inside the seed is significant. It shows conclusively that seed were impregnated with endrin and that it may be possible to introduce other chemical protectants into the seed without impairing viability.

Effectiveness of treated seed against mice varied by treatment. The 1.0 EC treatment was approximately twice as effective as the 0.5 EC coating, which was consistently the least effective of all treatments. Since endrin in the 0.5 EC seed was at the "recommended" level, and in absence of other published data on the treatment for comparison, the basis for the recommendation is rather difficult to understand. Although the original treatment (Anonymous 1956) called for 0.5 percent endrin and 2 percent Arasan,[6/] the latter chemical has generally been eliminated from the treatment in the Pacific Northwest. Elimination apparently resulted from one field test which indicated that the chemical did not significantly improve protection even though the data showed many more seedlings produced when it was added (Dick et al. 1958). It is conceivable, therefore, that poor performance of the 0.5 EC seed in our experiments was due to the absence of Arasan. This view is supported by our unpublished data[7/] indicating that Arasan significantly increases the effectiveness of the 0.5-percent coating treatment. The endrin-Arasan combination has been successfully used for many years on southern pines (Mann 1968).

There were no differences in effectiveness among the endrin impregnation treatments. Apparently, the two solvents allowed similar amounts of endrin to enter the seed, and increasing the soaking time with either solvent probably did not affect the endrin content. All impregnations were also as effective as the 1.0 EC coating, even though impregnated seed contained about half as much endrin, as is indicated by analysis of the EDIa seed. This was probably due to the presence of the chemical on and in the impregnated seed and only on the surface with the coating treatment.

Consumption by mice of impregnated seed (EDIa) did not change significantly after helicopter dispersal or after repeated washings with water, indicating that the seed can withstand sowing operations and weathering in the field. Further, reduced feeding by mice on the impregnated "endosperms" presents additional evidence for success of impregnations and suggests that this method of treating seed would be more effective than coating.

CONCLUSIONS

Endrin is still the only chemical used for direct application on coniferous seed to protect it from rodents. Interest in the possibility of protecting seed by using truly repellent chemicals rather than toxicants remains high, and

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6/ Tetramethylthiuram disulphide (TMTD).
7/ See footnote 5.

screening for repellents continues. However, until a true repellent becomes available, the endrin treatment as now used should be improved. It is evident from our results that doubling the endrin concentration increases the effectiveness of the treatment, but we question this approach in view of the potential hazards involved because of very well-known toxicity of the chemical. Certainly, impregnation with endrin would be more desirable. The method would provide far better protection than is now possible with the 0.5-percent coating treatment, with an essentially similar endrin concentration. In addition, endrin losses from seed during and after sowing and consequent contamination of the forest environment would appear less likely from impregnated seed. However, because of expected higher cost and complexity of impregnating compared with coating, use of additives which may increase the effectiveness of coating should also be considered.

LITERATURE CITED

Anonymous.
1956. 1956 formulation for the treatment of coniferous tree seed. U.S. Fish and Wildl. Serv., Wildl. Res. Lab., Denver, 2 pp. (Spec. release)

Association of Official Seed Analysts.
1965. Rules for testing seeds. Ass. Offic. Seed Anal. Proc. 54(2), 112 pp.

Dick, James, Finnis, J. M., Hunt, Lee O., and Kverno, Nelson B.
1958. Treatment of Douglas-fir seed to reduce loss to rodents. J. Forest. 56: 660-661.

Dimock, Edward J., II.
1957. A comparison of two rodent repellents in broadcast seeding Douglas-fir. USDA Forest Serv. Pacific Northwest Forest & Range Exp. Sta. Res. Pap. 20, 17 pp.

Edgren, James W.
1968. Potential damage to forest tree seed during processing, protective treatment, and dissemination. Pacific Northwest Forest & Range Exp. Sta. USDA Forest Serv. Res. Note PNW-89, 8 pp.

Hooven, Edward F.
1957. Field test of endrin-treated Douglas-fir seed. Oreg. Forest Lands Res. Center Res. Note 28, 8 pp.

Jones, LeRoy, and Havel, Kenneth.
1968. Effect of methyl bromide treatments on several species of conifer seed. J. Forest. 66: 858-860.

Mann, William F., Jr.
1968. Ten years' experience with direct-seeding in the South. J. Forest. 66: 828-833.

Snedecor, George W.
1961. Statistical methods applied to experiments in agriculture and biology. 534 pp. Ames: Iowa State Univ. Press.

Stein, William I.
1967. Laboratory tests--are they doing the job? West. Reforest. Coord. Comm. Proc. 1967: 20-23.

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Radwan, M. A., Crouch, G. L., and Ellis, W. D.
1970. Impregnating and coating with endrin to protect Douglas-fir seed from rodents. USDA Forest Serv. Res. Pap. PNW-94, 17 pp., illus. Pacific Northwest Forest & Range Experiment Station, Portland, Oregon.

Endrin was applied to seed by coating at two different levels and by impregnation using two different solvents. Effects of treatments on germination, endrin content, and seed consumption by deer mice were evaluated. Impregnation appears promising. However, research to improve effectiveness of coating seed should also be considered, if only for economic reasons.

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